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Extramedullary Relapse of Acute Leukemia after Haploidentical Hematopoietic Stem Cell Transplantation: Incidence, Risk Factors, Treatment, and Clinical Outcomes

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A B S T R A C T

We examined the incidence, risk factors, treatment, and clinical outcomes of extramedullary relapse (EMR) in 961 acute leukemia patients undergoing HLA-haploidentical hematopoietic stem cell transplantation (haplo-HSCT) between 2002 and 2013. Multiple control subjects were selected at random from the same cohort and matched to EMR cases for diagnosis, disease status at HSCT, age at the time of the HSCT, and year of HSCT. Forty patients exhibited EMR, with a median time to EMR of 207 days. The cumulative incidence of EMR was 4.0% at 3 years, and the incidence was higher in acute lymphoblastic leukemia patients compared with acute myeloid leukemia patients (5.6% versus 2.4%). In the multivariate analysis, non–complete remission (CR) status at HSCT (hazard ratio [HR] = 4.6; $P = .018$) and non–chronic graft-versus-host disease after HSCT (HR = 3.2; $P < .001$) were the independent risk factors for EMR after haplo-HSCT. Twenty-seven patients received combination treatments, and the proportion of patients who achieved CR was higher than those who received single treatment. Multifocal involvement at EMR (HR = 2.7; $P = .024$) and non-CR after EMR treatments (HR = 4.6; $P < .001$) were the independent risk factors for poor survival rates among EMR patients. We found that graft-versus-leukemia effect may help to prevent EMR after haplo-HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the most effective treatments for hematological malignancies, helping to achieve long-term remission and potentially curing many patients. Although significant progress has been made in allogeneic HSCT, post-transplant relapse remains one of the most important causes of transplant failure, and extramedullary relapse (EMR) accounts for a substantial part of total relapses. Some researchers have reported the incidence of EMR is .65% to 20% for patients who receive allogeneic HSCT [1,2].

Although risk factors and clinical outcomes for post-HSCT EMR have been described in acute leukemia patients, most studies enrolled HLA-identical sibling donors or HLA-unrelated donor HSCT recipients [3-5]. Considerable progress has been made in the field of HLA-haploidentical HSCT (haplo-HSCT) over the past 2 decades, and the haploidentical donor has become a promising alternative donor source for patients lacking an HLA-identical sibling donor. Despite the fact that haplo-HSCT may be associated with a potent graft-versus-leukemia (GVL) effect, some patients still develop EMR after transplantation. Yoshihara et al. [6] reported 9 haplo-HSCT recipients who developed post-HSCT EMR; however, the small sample size of EMR patients might preclude stable estimations. Thus, the characteristic patterns of EMR after haplo-HSCT remain unknown. Therefore, we used both a retrospective cohort study design and a nested case-control approach [7] to investigate the incidence, risk factors, treatment, and clinical outcomes of EMR after haplo-HSCT.

METHODS

Patients

A total of 961 consecutive patients underwent haplo-HSCT for acute leukemia between January 2002 and February 2013 at the Institute of Hematology, Peking University. Medical records maintained at the Institute were the primary source of data for the current study. Patients who were

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Table 1
Characteristics of AML Patients

Characteristics	Control Group (n = 70)	EMR Group (n = 14)	*P*
Median age at HSCT, yr (range)	26 (9-49)	27 (10-48)	.513
Gender, n (%)			
Male	51 (72.9)	8 (57.1)	.336
Female	19 (27.1)	6 (42.9)	
Disease status at transplantation, n (%)			
CR	65 (92.9)	13 (92.9)	1.000
Non-CR	5 (7.1)	1 (7.1)	
Poor cytogenetic risk, n (%)	7 (10.0)	2 (14.3)	.641
Hyperleukocytosis at diagnosis, n (%)	11 (15.7)	1 (7.1)	.681
EM leukemia before HSCT, n (%)	5 (7.1)	3 (21.4)	.125
Chemotherapy resistance, n (%)	35 (50.0)	4 (28.6)	.142
Donor–recipient gender match, n (%)			
Male–male	27 (38.6)	5 (35.7)	.165
Male–female	11 (15.7)	1 (7.2)	
Female–male	24 (34.3)	3 (21.4)	
Female–female	8 (11.4)	5 (35.7)	
Donor–recipient relation, n (%)			
Father–child	17 (24.3)	4 (28.6)	.224
Mother–child	13 (18.6)	5 (35.7)	
Sibling–sibling	35 (50.0)	4 (28.6)	
Child–parent	1 (1.4)	1 (7.1)	
Other	4 (5.7)	0 (.0)	
Number of HLA-A, -B, -DR mismatches, n (%)			
1	6 (8.6)	3 (21.4)	.160
2	29 (41.4)	3 (21.4)	
3	35 (50.0)	8 (57.2)	
Grafts, n (%)			
BM + peripheral blood	69 (98.6)	14 (100.0)	1.000
BM	1 (1.4)	0 (.0)	
Conditioning regimen, n (%)			
Chemotherapy	69 (98.6)	14 (100.0)	1.000
TBI + chemotherapy	1 (1.4)	0 (.0)	
Acute GVHD, n (%)			
Negative	27 (38.6)	7 (50.0)	.563
Grades I-II	39 (55.7)	6 (42.9)	
Grades III-IV	4 (5.7)	1 (7.1)	
cGVHD			
Negative	32 (45.7)	10 (71.4)	.231
Limited	16 (22.9)	2 (14.3)	
Extensive	22 (31.4)	2 (14.3)	
Year of HSCT, n (%)			
Before 2009	34 (48.6)	4 (28.6)	.170
2009 or later	36 (51.4)	10 (71.4)	
Median mononuclear cells, $\times 10^8$/kg (range)	7.8 (4.2-13.7)	7.8 (5.7-10.4)	.784
Median $CD34^+$ counts, $\times 10^6$/kg (range)	2.2 (.3-7.9)	2.9 (1.0-8.3)	.282
Median duration of follow-up, yr (range)	2.2 (.2-9.6)	1.3 (.7-2.8)	.052

None of the EMR patients was diagnosed as acute mixed lineage leukemia. The criterion for statistical significance was $P < .05$.

Table 2
Characteristics of ALL Patients

Characteristics	Control Group (n = 126)	EMR Group (n = 26)	*P*
Median age at HSCT, yr (range)	18 (3-56)	18 (2-53)	.321
Gender, n (%)			
Male	79 (62.7)	17 (65.4)	.796
Female	47 (37.3)	9 (34.6)	
Disease status at transplantation, n (%)			
CR	110 (87.3)	22 (84.6)	.751
Non-CR	16 (12.7)	4 (15.4)	
Poor cytogenetic risk, n (%)	36 (28.6)	6 (23.1)	.568
Hyperleukocytosis at diagnosis, n (%)	44 (34.9)	6 (23.1)	.242
EM leukemia before HSCT, n (%)	18 (14.3)	8 (30.8)	.081
Chemotherapy resistance, n (%)	41 (32.5)	6 (23.1)	.342
Donor–recipient gender match, n (%)			
Male–male	37 (29.4)	12 (46.2)	.414
Male–female	23 (18.2)	4 (15.4)	
Female–male	39 (31.0)	5 (19.2)	
Female–female	27 (21.4)	5 (19.2)	
Donor–recipient relation, n (%)			
Father–child	44 (34.9)	15 (57.7)	.165
Mother–child	49 (38.9)	7 (26.9)	
Sibling–sibling	29 (23.0)	3 (11.5)	
Child–parent	2 (1.6)	1 (3.9)	
Other	2 (1.6)	0 (.0)	
Number of HLA-A, -B, -DR mismatches, n (%)			
1	14 (11.1)	4 (15.4)	.677
2	49 (38.9)	8 (30.8)	
3	63 (50.0)	14 (53.8)	
Grafts, n (%)			
BM + peripheral blood	123 (97.6)	26 (100.0)	1.000
Peripheral blood	2 (1.6)	0 (.0)	
BM	1 (0.8)	0 (.0)	
Conditioning regimen, n (%)			
Chemotherapy	120 (95.2)	23 (88.5)	.183
TBI + chemotherapy	6 (4.8)	3 (11.5)	
Acute GVHD, n (%)			
Negative	38 (30.2)	9 (34.6)	.346
Grades I-II	69 (54.8)	16 (61.5)	
Grades III-IV	19 (15.1)	1 (3.9)	
cGVHD			
Negative	52 (41.3)	15 (57.7)	.305
Limited	32 (25.4)	5 (19.2)	
Extensive	42 (33.3)	6 (23.1)	
Year of HSCT, n (%)			
Before 2009	56 (44.4)	12 (46.2)	.873
2009 or later	70 (55.6)	14 (53.8)	
Median mononuclear cells, $\times 10^8$/kg (range)	7.8 (3.0-17.2)	7.8 (3.9-10.9)	.688
Median $CD34^+$ counts, $\times 10^6$/kg (range)	2.5 (.6-9.4)	2.6 (.8-7.7)	.487
Median duration of follow-up, yr (range)	1.2 (.1-10.3)	1.3 (.1-8.9)	.811

None of the EMR patients was diagnosed as acute mixed lineage leukemia. The criterion for statistical significance was $P < .05$.

free of extramedullary (EM) leukemia disease or whose EM leukemia had achieved complete remission (CR) before HSCT and who developed EMR after HSCT were considered as EMR cases. For each EMR patient, a set of control subjects (4 to 5) was randomly selected from the same cohort at the time at which EMR occurred ("risk-set sampling") [8] and was matched according to the following criteria: diagnosis, disease status at HSCT (CR or non-CR), age at the time of the HSCT (±5 years), and year of the HSCT (±2 years). Forty patients with EMR and 196 matched control subjects were included in the analysis; 36 EMR patients were matched with 5 control subjects and the other 4 patients were matched with 4 control subjects (Tables 1 and 2). The study protocol was approved by the ethics committee at Peking University People's Hospital. Informed consent was obtained according to the Declaration of Helsinki.

Transplant Regimens

The major preconditioning treatment consisted of cytarabine (4 g/[m^2·day] for 2 days), busulfan (4 mg/[kg·day] administered orally for 3 days before January 2008 or 3.2 mg/[kg·day] administered intravenously for 3 days after January 2008), cyclophosphamide (1.8 g/[m^2·day] for 2 days), and simustine (250 mg/m^2 for 1 day), along with rabbit antithymocyte globulin (ATG; thymoglobulin; rabbit ATG from Imtix Sangstat, Lyon, France; 2.5 mg/[kg·day] for 4 days). Some patients (n = 10) also received total body irradiation (TBI) as part of the conditioning regimens. The granulocyte colony-stimulating factor mobilized, fresh, and unmanipulated bone marrow and/or peripheral blood harvests were infused into the recipients on the day of collection. Granulocyte colony-stimulating

factor (5 to 10 μg/kg per day s.c.) was provided to all recipients from day 6 after transplantation until their WBC counts exceeded 2×10^9 cells/L for 3 consecutive days. Additionally, all patients received cyclosporine A, mycophenolate mofetil, and short-term methotrexate for graft-versus-host disease (GVHD) prophylaxis [9]. Prophylactic, interventional, and therapeutic donor lymphocyte infusion (DLI) after transplantation was performed as previously reported [10]. Prophylaxis of central nervous system (CNS) leukemia before HSCT consisted of intrathecal chemotherapy for at least 6 doses for acute lymphoblastic leukemia (ALL) patients, at least 4 doses for acute myeloid leukemia (AML) patients with WBC counts $> 100 \times 10^9$ cells/L at diagnosis, and at least 2 doses for other AML patients during induction chemotherapy and consolidation chemotherapy.

Donor Selection and HLA Typing

Patients without a suitable closely HLA-matched unrelated donor, namely, with >8 of 10 matching HLA-A, -B, -C, -DR, and -DQ loci, and 5 of 6 or 6 of 6 matching HLA-A, -B, and DR loci, were eligible for haplo-HSCT if an HLA-identical sibling donor was unavailable as a first treatment option. To determine HLA-A and HLA-B status, low-resolution DNA techniques were used. High-resolution techniques were used for HLA-DRB1 typing. Each patient with a haploidentical related donor received stem cells from a family member who shared 1 HLA haplotype with the patient but differed to a variable degree for the HLA-A, -B, and -D antigens of the haplotype not shared. Apart from each donor–recipient pair, HLA typing was performed for parents and offspring to be strictly analyzed to guarantee true haploid genetic background [11].

Definitions

EMR included cases of both isolated EMR and concurrent bone marrow relapse (BMR), and BMR was defined as the reappearance of blasts in the peripheral blood or more than 5% blasts in the BM smear. In isolated EMR patients, the evaluation of BM status (including morphology, cytogenetics, and molecular examination) had to reveal CR and chimerism study had to reveal full-donor chimerism. EMR was identified on physical examination and/or by imaging studies (computed tomography, magnetic resonance imaging, or positron emission tomography), and pathological confirmation was performed whenever possible but was not necessarily required. CNS relapse was diagnosed when leukemic cells were identified in the cerebrospinal fluid.

Adverse cytogenetics was defined as del(5q) or monosomy 5; monosomy 7 or del(7q); abnormal 3q, 9q, 11q, 21q, or 17p; t(6;9); t(9;22); and complex karyotypes in AML [12] and was defined as t(9;22), t(4;11), t(8;14), low hypodiploidy-near triploidy, or complex karyotype in ALL [13]. Hyperleukocytosis in cases of AML was defined as a peripheral WBC count of over 100×10^9 cells/L at diagnosis, B-precursor ALL as a count of over 30×10^9 cells/L, and T-precursor ALL as a count of over 100×10^9 cells/L.

Chemotherapy resistance before HSCT was defined as remission requiring more than 1 course of induction therapy or relapse during consolidation chemotherapy and inability to achieve CR after reinduction therapy. The diagnosis of GVHD was made in accordance with accepted international criteria [14,15]. For isolated EMR patients, CR was defined as disappearance of all clinical signs of EM leukemia, confirmed by physical examination and imaging studies. Particularly for CNS relapse patients, CR was defined as disappearance of leukemia cells in cerebrospinal fluid. For EMR with concurrent BMR patients, CR was defined as presence of less than 5% blast cells in normocellular BM, peripheral blood cell counts showing at least 1.5×10^9 neutrophils/L, and disappearance of all clinical signs of EM leukemia. Overall survival (OS) was defined as the time from transplantation to death from any cause.

Statistical Analysis

In the cohort analysis, competing risk analysis was performed to calculate the cumulative incidence of EMR [16], treating death without EMR as the competing event for EMR. The time to risk was computed from the date of haplo-HSCT to the date of onset of EMR, the date of last contact, or the date of death, whichever came first. The log-rank test was used to compare the various subpopulations.

In the nested case-control analysis, continuous variables were compared using the Mann-Whitney U test, and categorical variables were compared using chi-square and Fisher's exact tests. Survival probabilities were estimated using the Kaplan-Meier method. Hazard ratios (HRs) for EMR were estimated from univariate and multivariate competing risk regression analyses [17], and factors included in the regression model were age, sex, diagnosis, adverse cytogenetics, hyperleukocytosis, chemotherapy resistance before HSCT, a history of EM leukemia before HSCT, disease status at transplantation (CR versus non-CR), HLA disparity (1 locus versus ≥2 loci), donor–recipient gender matching (female–male versus others), acute GVHD, and chronic GVHD (cGVHD). HRs for OS of EMR patients were estimated from univariate and multivariate Cox regression analyses. The factors studied in the Cox regression model were multifocal involvement at EMR (≥2 versus 1), therapy regimen for EMR (combination versus single treatment), GVHD after therapy (yes versus no), and achievement of CR after therapy (yes versus no).

All factors with $P < .1$ in the univariate analysis were included in the multivariate regression, and $P < .05$ was considered to be statistically significant. All reported P values were based on 2-sided hypothesis tests. Data analyses were primarily conducted using SPSS software (SPSS Inc., Chicago, IL), whereas the R software package (version 2.6.1; http://www.r-project.org) was used for competing risk analysis.

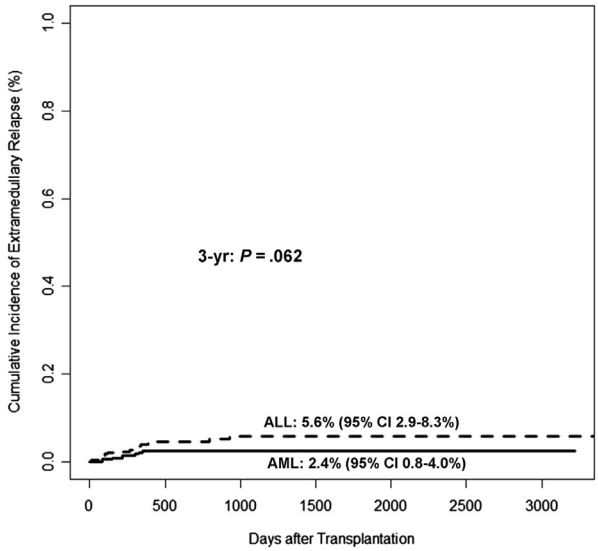

Figure 1. Cumulative incidence of EMR for AML and ALL patients.

RESULTS

Incidence and Characteristics of EMR

During the follow-up period, 40 patients exhibited EMR with a median time from HSCT to EMR of 207 days (range, 10 to 2809), and the detail of each EMR case is described in Supplementary Table 1. Fourteen patients had isolated EMR, and 26 developed EMR with concurrent BMR. Of these 26 cases, 11 patients who initially had EMR later developed BMR, 5 patients with BMR later developed EMR, and the remaining 10 patients experienced EMR and BMR almost simultaneously.

The 3-year cumulative incidence of EMR was 4.0% (95% confidence interval [CI], 2.4% to 5.6%). The 3-year cumulative incidences of isolated EMR and EMR with concurrent BMR were 1.8% (95% CI, .8% to 2.8%) and 2.2% (95% CI, 1.8% to 2.6%), respectively. The 3-year cumulative incidence of ALL patients was 5.6% (95% CI, 2.9% to 8.3%), which was higher than that of AML patients (2.4%; 95% CI, .8% to 4.0%; $P = .062$) (Figure 1).

The EM sites involved in the relapse varied, including CNS (n = 19), bone (n = 5), testis (n = 4), breast (n = 4), soft tissue (n = 4), lymph nodes (n = 4), mediastinum (n = 4), peritoneum (n = 2), ovary (n = 1), and gastrointestinal tract (n = 1). Multifocal involvement at EMR (≥2) was observed in 8 patients. Eleven patients had a history of EM leukemia before HSCT, and 6 of them relapsed in the same site as the previous leukemia.

Risk Factors of EMR

For overall EMR of acute leukemia patients, non-CR status at transplantation (HR = 4.6; 95% CI, 1.3 to 16.3, $P = .018$) and non-cGVHD after transplantation (HR = 3.2; 95% CI, 1.7 to

Table 3
Univariate and Multivariate Analyses of the Factors associated with EMR

Variable	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age						
<Median	1.0		.597			
≥Median	.8	.4-1.7				
Sex						
Male	1.0		.862			
Female	.9	.5-1.9				
Diagnosis						
AML	1.0		.806			
ALL	1.1	.5-2.4				
Adverse cytogenetics						
No	1.0		.941			
Yes	1.0	.4-2.3				
Hyperleukocytosis						
No	1.0		.293			
Yes	.6	.3-1.5				
Chemotherapy resistance before HSCT						
No	1.0		.390			
Yes	2.4	.3-17.9				
EM leukemia before HSCT						
No	1.0			1.0		.060
Yes	3.0	.9-9.7	.062	3.7	.9-14.6	
Disease status at HSCT						
CR	1.0		**.016**	1.0		**.018**
Non-CR	4.8	1.3-16.9		4.6	1.3-16.3	
HLA disparity						
1 locus	1.0		.118			
≥2 loci	.5	.2-1.2				
Donor–recipient gender matching						
Others	1.0		.123			
Female–male	.5	.2-1.2				
Acute GVHD						
Yes	1.0		.367			
No	1.3	.7-2.5				
cGVHD						
Yes	1.0		**.003**	1.0		**<.001**
No	2.9	1.5-5.9		3.2	1.7-6.2	

Bold font indicates statistical significance, and the criterion for statistical significance was $P < .05$.

6.2; $P < .001$) were the independent risk factors for EMR after haplo-HSCT in multivariate analysis (Table 3). For ALL patients, non-CR status at transplantation (HR = 6.1; 95% CI, 1.1 to 34.6; $P = .042$), a history of EM leukemia before transplantation (HR = 3.8; 95% CI, 1.5 to 9.2; $P = .004$), and non-cGVHD after transplantation (HR = 2.9; 95% CI, 1.3 to 6.5; $P = .009$) were the independent risk factors for EMR in multivariate analysis. For AML patients, only non-cGVHD after transplantation was found to be independently associated with EMR (HR = 4.5; 95% CI, 1.2 to 16.4; $P = .023$) in multivariate analysis.

Comparison between EMR and BMR

In the control group, 46 patients experienced isolated BMR with a median time from HSCT to isolated BMR of 210 days (range, 47 to 1392). The proportion of patients who were chemotherapy resistant before HSCT was significantly lower in EMR group as compared with the isolated BMR group (25.0% versus 65.2%, $P < .001$), whereas the other variables were comparable between the EMR and isolated BMR group (Supplementary Table 2).

One EMR patient (2.5%) and 6 isolated BMR patients (13.0%) received prophylactic DLI before relapse ($P = .116$); 7 EMR patients (17.5%) and 6 isolated BMR patients (13.0%) who were minimal residual disease positive after HSCT received interventional DLI ($P = .565$). The 3-year probabilities of OS in cases of isolated EMR, EMR with concurrent BMR, and isolated BMR groups were comparable (Figure 2).

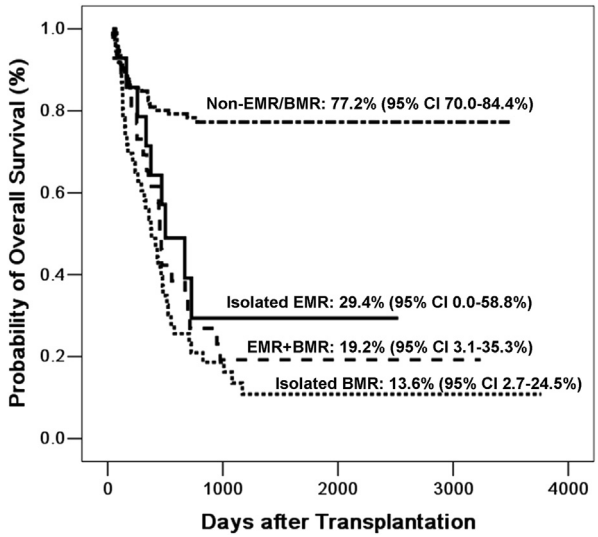

Figure 2. Kaplan-Meier estimates of overall survival: $P = .523$ (isolated EMR versus EMR+BMR), $P = .169$ (isolated EMR versus isolated BMR), $P = .355$ (EMR+BMR versus isolated BMR); $P = .001$ (isolated EMR versus non-EMR/BMR), $P < .001$ (isolated BMR versus non-EMR/BMR), and $P < .001$ (EMR+BMR versus non-EMR/BMR).

Treatment and Clinical Outcomes of EMR Patients

Two EMR patients refused any treatment and died of relapse. Nineteen patients who developed CNS relapse were all treated with intrathecal chemotherapy; in addition, 10 of these patients were also treated with cranial irradiation, and 8 patients with concurrent BMR received systematic chemotherapy and DLI. Among another 19 patients, 13 received systematic chemotherapy, 12 received DLI, 10 received irradiation, and 3 received local surgery. Twenty-seven patients received combination treatments (≥2 types of treatment), and the proportion of patients who achieved CR was higher than those who received single treatment (63.0% versus 36.4%, $P = .167$), although the difference was not statistically significant. Twelve patients developed GVHD after treatment for EMR (skin, n = 10; gastrointestinal tract, n = 6; liver, n = 3; oral cavity, n = 2), and the proportion of patients who achieved CR was significantly higher in patients who developed GVHD compared with those without GVHD (83.3% versus 39.3%, $P = .011$).

Twenty-one EMR patients achieved CR after treatment. To date, 9 of them still survive, whereas the other 12 died (9 died of leukemia recurrence, 2 died of infection, and 1 died of GVHD). In multivariate analysis, multifocal involvement at EMR (HR = 2.7; 95% CI, 1.1 to 6.4; $P = .024$) and non-CR after EMR treatments (HR = 4.6; 95% CI, 2.0 to 10.5; $P < .001$) were the independent risk factors for poor OS among EMR patients.

DISCUSSION

Although several studies have identified the characteristics of post-HSCT EMR, to the best of our knowledge, this study is the largest reported series of EMR after haplo-HSCT in acute leukemia patients. We observed the 3-year cumulative incidence of EMR was 4% after haplo-HSCT, and the

incidence of EMR seems to be higher in ALL patients than that in AML patients. In addition, our analyses also revealed that cGVHD can decrease the risk of EMR in haplo-HSCT recipients, and development of GVHD after treatment of EMR may help to achieve CR.

Several studies have identified the correlation between cGVHD and EMR after HSCT; however, results were controversial. Some authors observed a higher incidence of cGVHD in patients with EMR compared with BMR patients [18], and occurrence of EMR was higher in patients suffering from extensive cGVHD [19]. Other authors reported the incidence of EMR did not differ between patients with cGVHD and patients without cGVHD, and cGVHD could not decrease the risk of EMR [2,4,5,20]. Some of the EM sites, such as the CNS and testis, may act as "sanctuary" locations for leukemic cells, thus providing protection from both cytotoxic conditioning regimens and immune surveillance through GVL effects [4,5]. Cytotoxic $CD8^+$ T cells, the main mediators of the GVL effect, are much more concentrated in the BM compared with that in peripheral tissues [18]. In addition, recruitment of accessory cells necessary to achieve efficient local antileukemic activity may be deficient in sites of EMR [20]. However, some authors reported that acute leukemia patients who develop EMR after HSCT were treated with DLI, developed GVHD, and achieved complete resolution [21-23], and these reports provided evidence for the GVL effects toward EMR. In our studies, we found that cGVHD can decrease the risk of EMR in haplo-HSCT recipients. In addition, we observed the occurrence of GVHD after treatment for EMR can help to achieve CR, and EMR patients who achieved CR had better OS. These are interesting results that suggest the potent GVL effect after haplo-HSCT might be responsible for better eradication of EM leukemia cells.

Several studies have reported adverse cytogenetics, hyperleukocytosis at diagnosis, male gender, TBI, and the source of peripheral blood stem cell (PBSC) as risk factors for EMR after HSCT [2-5]; however, in our study we did not observe the correlation between these variables and occurrence of EMR. We had reported that adverse cytogenetics and hyperleukocytosis were not associated with relapse after haplo-HSCT [24], which possibly suggests haplo-HSCT may help to overcome the poor prognostic significance of these factors at diagnosis in acute leukemia patients. Ge et al. [2] observed that male gender had a high frequency of EMR and there was a relatively high incidence of EMR with testis leukemia subtype in the cohort (5/26); however, only 10% of patients developed testis leukemia in our cohort. Although some authors observed that patients who received TBI or PBSCs had a higher risk of EMR, these patients had other risk factors for EMR (such as advanced stage of the disease and a history of EM leukemia before HSCT) [2], and other studies failed to observe the correlation between these 2 variables and EMR [4,18,25]. In our study the number of patients who received TBI or PBSCs was too small to permit further investigation of the correlation between these 2 variables and EMR. Consequently, we could not completely exclude the influence of TBI and PBSCs on post-HSCT EMR.

There have been few studies on the correlation between chemotherapy resistance before HSCT and post-HSCT EMR. In our study, we observed the proportion of patients who were chemotherapy resistant was significantly lower in EMR groups compared with that in isolated BMR groups. This suggests that although some patients were evaluated as having achieved CR before HSCT, asymptomatic EM sites might not be routinely studied clinically, and hence, EM leukemia before HSCT may be underreported. We also found that a history of EM leukemia before transplantation seems more common in EMR group. Therefore, it is important to establish new methods with higher sensitivity that can detect the presence of EM leukemia better before HSCT.

Although Lee et al. [3] observed no significant difference in postrelapse survival between patients with isolated BMR and EMR with or without BM involvement, other authors observed that survival was significantly better in patients with isolated EMR [4-6,18]. We also observed a better prognosis in patients with isolated EMR compared with those with BMR or systemic relapse, and the trend suggested the difference would have been more significant if more patients were studied. Some authors suggested that in case of isolated EMR, persistence of donor hematopoiesis in the recipient's BM may be responsible for increased susceptibility to treatments [5]. Thus, the strategy to prevent systemic relapse is important to improve the outcomes.

There has been no standardized therapeutic strategy for post-HSCT EMR. Although several studies have shown that local radiotherapy could offer some patients long-term survival, most patients developed systemic relapse [26]. Shi et al. [5] observed patients treated with combination therapy involving local surgery, radiotherapy, chemotherapy, and DLI achieved longer survival compared with those who received single therapy. However, Harris et al. [4] did not observe the advantage of combination strategies based on chemotherapy. In our study, although the combination treatment seemed to increase the CR rate, it could not improve OS. Some authors suggested that systemic chemotherapy may also abrogate the effector cells on GVL [6]; in addition, the infection and GVHD may lead to nonrelapse mortality. Thereafter, some authors suggested that gemtuzumab ozogamicin [27] or hypomethylating agents [28] may further improve the clinical outcomes of post-HSCT EMR patients, but it should be confirmed by further studies.

ATG plays a critical role in our protocol of GVHD prophylaxis; however, we also observe that cGVHD may help to decrease the risk of EMR. Because all patients received ATG for GVHD prophylaxis in this study, we did not know whether ATG increases the risk of sanctuary site relapse. However, data from 6 studies including 568 patients suggested the incidence of relapse was not significantly different between ATG and non-ATG groups [29]. The protective effect of ATG on cGVHD included the limited form, but more so the extensive form, of the disease [30], and some authors observed that only limited cGVHD was associated with lower relapse rates and improved survival [31]. In addition, some authors also observed the antileukemic activity of ATG by measuring apoptosis in myeloid and lymphatic leukemia cell lines and primary leukemia cells [32]. Thus, no evidence suggested that ATG may increase the risk of EMR after haplo-HSCT. Further studies including haplo-HSCT recipients receiving other protocols of GVHD prophylaxis (such as post-transplantation high-dose cyclophosphamide) may help to further investigate the influence of ATG on post-HSCT EMR.

This study has certain limitations. There were only 40 EMR events, and this relatively small number may not provide sufficient statistical power to prove the positive effect of cGVHD on EMR. Therefore, it would be premature to derive the conclusion that cGVHD could decrease the risk of EMR after haplo-HSCT. A multicenter trial with a larger population may be needed to confirm the characteristics of patients who develop EMR after haplo-HSCT.

In summary, we found that 3-year cumulative incidence of EMR was 4.0% in haplo-HSCT recipients, and non-CR status

at transplantation and non-cGVHD after transplantation were the independent risk factors for EMR after haplo-HSCT. In addition, the GVL effect may help to prevent and eradicate the EMR after haplo-HSCT, but this needs to be confirmed by further studies.

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SUPPLEMENTARY DATA

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